Sequencing Procyanidin Oligomers by Fast Atom Bombardment Mass Spectrometry

Sir: Polymeric procyanidins (condensed tannins) are present in a wide distribution of plants, occurring in particularly high concentrations in some barks, leaves, and fruits (*1*). These phenolic polymers complex with proteins and therefore inhibit enzyme activity (*2*), are important contributors to the flavor of foods (*3, 4*), and influence the nutritional value of plants (*5, 6*). Procyanidins are also credited with a role in protecting plants from microorganisms and insects (*7*,

8). As abundant constituents of the barks of many commercially harvested timber species, procyanidins are potential replacements for petroleum-derived, phenolic polymers used industrially as adhesives, dispersants, and ion exchange materials (*9–11*). Although these polymers have been used throughout recorded history in applications such as leather tanning (*12*), little was known about their chemistry until recently. Current research is for the most part focused on their biogenesis, structure, and reactions (*13–19*).

Both ^1H and ^{13}C NMR spectroscopy have been primary tools for elucidating procyanidin structures. However, resonance multiplicity and broadening associated with rotational and conformational isomerism often severely complicate the interpretation of NMR spectra. Although remarkable advances have been made in elucidating the structures of these polymers over the past 15 years, questions remain about the occurrence and extent of branching, the relative proportions of different interflavanoid linkage types, and the sequential order of different monomer units in the polymers.

A growing body of literature (*20, 21*) advocates the use of fast atom bombardment mass spectrometry (FAB-MS) coupled with some mode of tandem mass spectrometry (MS–MS) (*22, 23*) as a powerful tool for partial or complete sequencing of biopolymers. This correspondence describes the sequencing of procyanidin oligomers by FAB-MS and linked scanning MS–MS. The abundant sequence ions observed can also be used to differentiate the two types of polymeric linkages found in procyanidins as well as to distinguish a branched trimer from linear isomers. Metastable decomposition pathways leading to the sequence ions are established for each compound.

EXPERIMENTAL SECTION

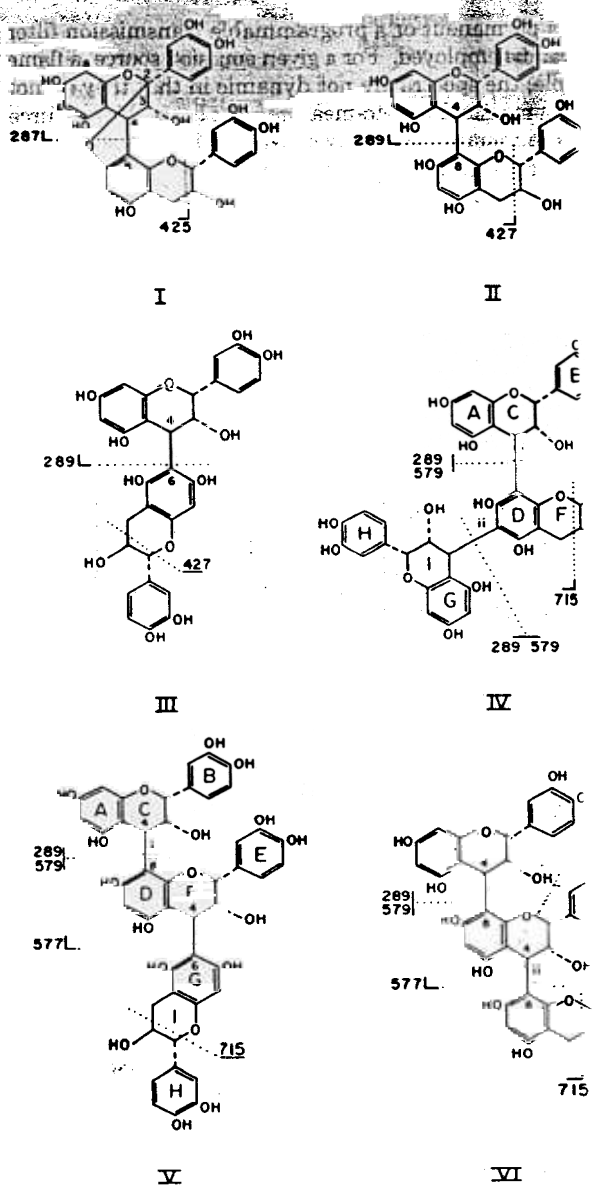
Procyanidin dimer A-1, epicatechin(4β→8;2β→*O*→7)catechin (I), was isolated from Arachis *hypogea* (*24*). The dimeric procyanidins B-1, epicatechin(4β→8)catechin (II), and B-7, epicatechin(4β→6)catechin (III), were made by acid-catalyzed cleavage of condensed tannins isolated from *Pinus taeda* bark in the presence of excess (+)-catechin (*16*). The branched trimer epicatechin(4β→8)catechin(4β→6)epicatechin (IV) was synthesized by reacting III with epicatechin-4β-phenyl sulfide at pH 9.0 and ambient temperature (*15, 17*). The linear trimers epicatechin(4β→8)epicatechin(4β→6)catechin (V) and epicatechin-(4β→8)epicatechin(4β→8)catechin (VI) were isolated from the phloem of *Pinus taeda* (*25*).

Mass spectra were obtained with a VG 7070E-HF mass spectrometer. A standard VG FAB source equipped with an Ion Tech saddle field atom gun was used. The sample compounds were dissolved in droplets of the FAB liquid matrix, a 5:1 mixture of dithiothreitol and dithioerythritol, directly on the target of the sample insertion probe. The samples were bombarded with 8 keV xenon atoms; the resulting secondary ions were accelerated from the source to 6 keV and mass analyzed at low resolution (1500–2000, 10% valley definition) with a scan rate of 10 s per mass decade. Metastable decomposition pathways were established with B/E and B^2/E linked scanning without collisional activation.

RESULTS AND DISCUSSION

The positive ion mass spectra produced by FAB-MS of the procyanidin oligomers (I–VI) all exhibit abundant $(M + H)^+$ ions; fragmentation occurs predominantly by cleavage of interflavanoid bonds to produce sequence ions. Retro-Diels–Alder (RDA) fission of the flavanoid monomer's heterocyclic ring system, similar to that recently reported for several flavonols (*26*), is also observed but to a lesser degree than the bond cleavages.

The dimers I, II, and III represent the two types of interflavanoid bonds found in procyanidins: the A-type (C4→C8;2β→*O*→7) double linkage as shown in the dimer A-1 (I) and the B-type (C4→C8 or C4→C6) carbon-to-carbon linkage as shown in dimers B-1 (II) and B-7 (III), respectively.



Mass peaks corresponding to $(M + H)^+$ and $(M - H_2$ ions for I and III are observed at m/z 577/559 and 5 respectively (Figure 1); fragmentation characterist interflavanoid bonds is readily apparent in the major i at m/z 287 for I and 289 for III. The spectrum o shown, is qualitatively similar to that of III; the sl ferences in relative abundances observed betwee sponding peaks in the mass spectra of II and III are due to the different locations of their interflavanoi Ion peaks attributable to RDA fission and subseque loss are observed at m/z 425 and 407 for I and m/z 409 for II and III.

It is well documented that B-type procyanidin which readily undergo acid-catalyzed cleavage to gi nium ions at the C4 position of the upper flavanoid *27*), are very acid labile in solution and that A-type which resist acid-catalyzed cleavage (*24, 28*), are n phase decompositions of FAB-desorbed ions are k mimic reactions in solution (*20, 21*); this is indeed with the two types of procyanidin dimers. Linked-sca from $(M + H)^+$ and its principal daughter ions show m/z 287 from I and m/z 289 from II and III can re gas-phase, unimolecular decompositions of the par However, in parallel to the reactions in solution c the metastable decomposition pathways leading t teristic sequence ions from the dimers are distinctly

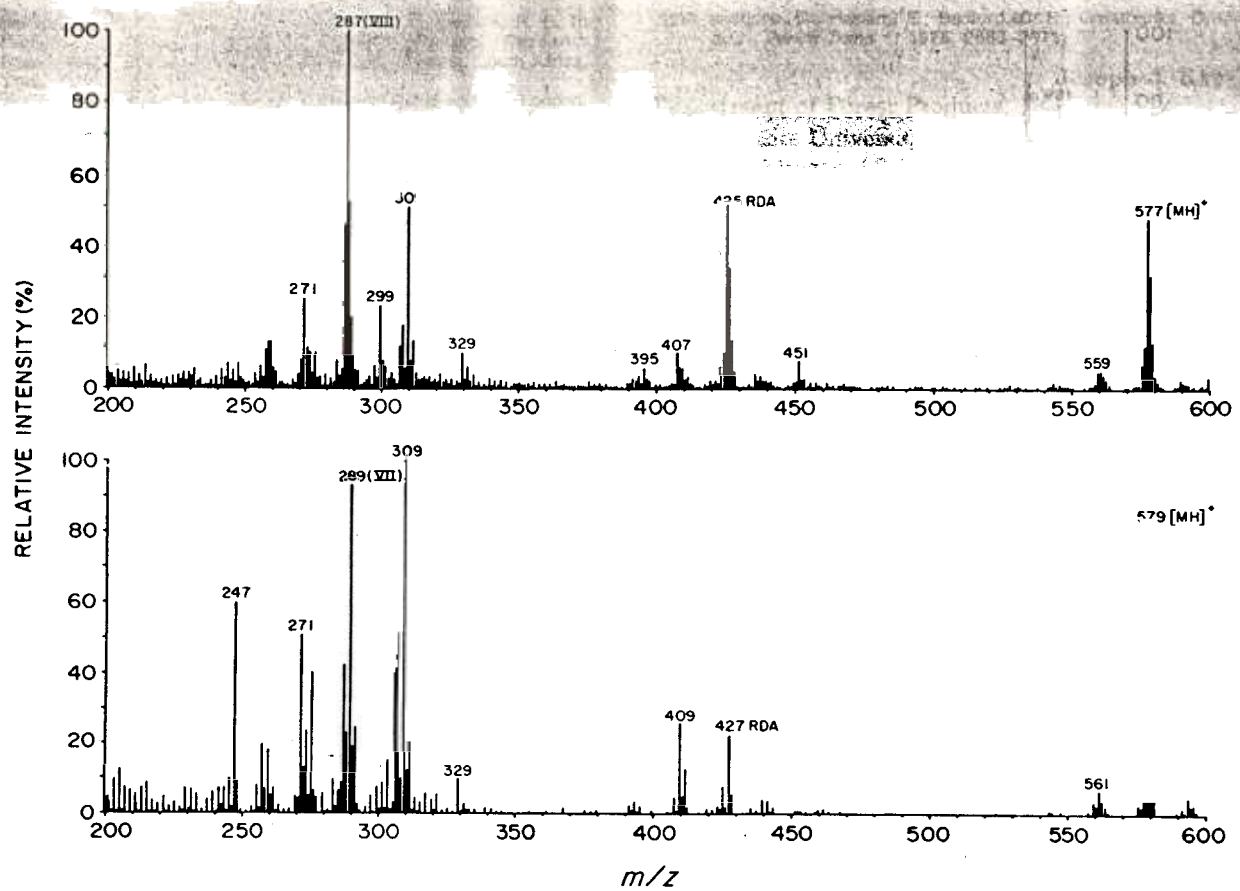
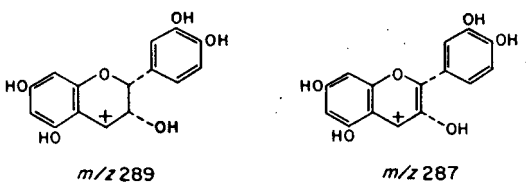
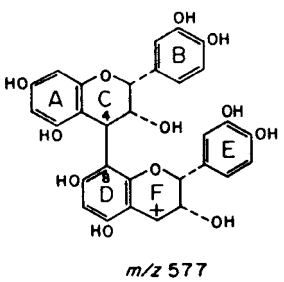


Figure 1. FAB mass spectra of procyanidin dimers (m/z 309 is a matrix ion peak): A-1 (I), upper spectrum; B-7 (III), lower spectrum.

for the two types of interflavanoid bonds (Figure 2A). In the case of B-type dimers, II and III, both B/E and B^2/E linked scans clearly show that the monomer ion at m/z 289 forms directly from the $(M + H)^+$ ion; the process can be pictured as simple protonation of the aromatic ring at the bonding carbon (C8 or C6) followed by bond cleavage to give the carbonium ion. By contrast, the A-1 dimer undergoes a two-step decomposition: RDA fission of the protonated parent ion, $(M + H)^+$, gives the ion at m/z 425, which subsequently decomposes to the monomer ion, m/z 287 (VIII).





The trimers IV–VI all give $(M + H)^+$ ion peaks at m/z 867 (Figure 3). Fragmentation of the upper interflavanoid linkage, i, in each leads to ion peaks at m/z 289 and 579. As in the

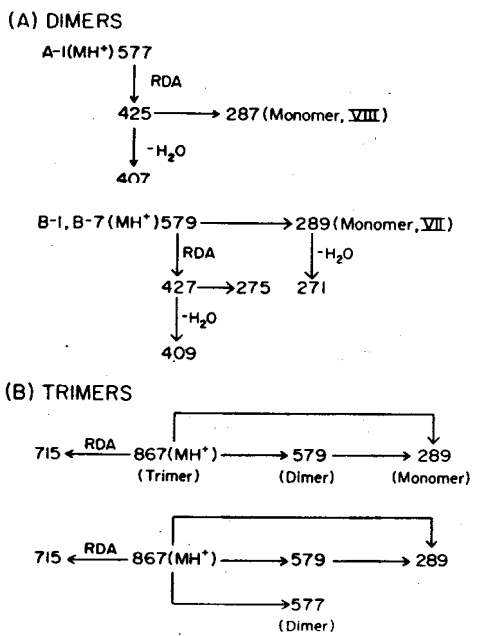


Figure 2. Metastable decomposition pathways leading to major sequence ions for procyanidin (A) dimers [A-1 (I), upper; B-1 and B-7 (II and III), lower] and (B) trimers [branched trimers (IV), upper; linear trimers (V and VI), lower].

case of the B-type dimers (II and III), the m/z 289 peak corresponds to the upper flavanoid unit, which can form a carbonium ion at C4 upon bond cleavage (VII). The m/z 579 ion peak corresponds to a protonated dimer moiety from the lower two flavanoid units.

However, fragmentation of the lower interflavanoid bond, ii, in the three trimers leads to different sequence ions depending on whether the trimer is branched or linear. In the

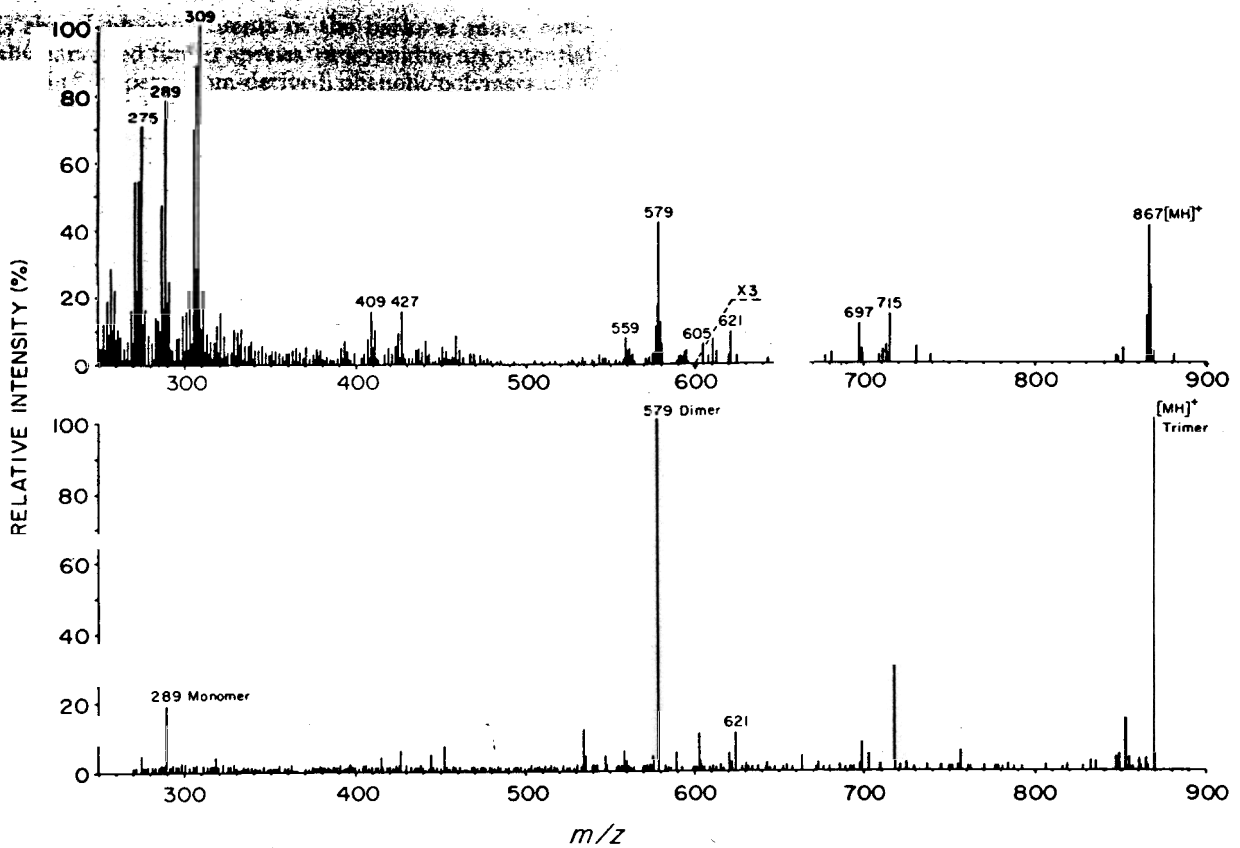


Figure 3. FAB mass spectrum of branched procyanidin trimer (IV) showing sequence ions at m/z 289 and 579 (upper spectrum); daughter ion linked scan showing unimolecular gas phase decomposition of $(M + H)^+$ ion (m/z 867) from (IV) above (lower spectrum).

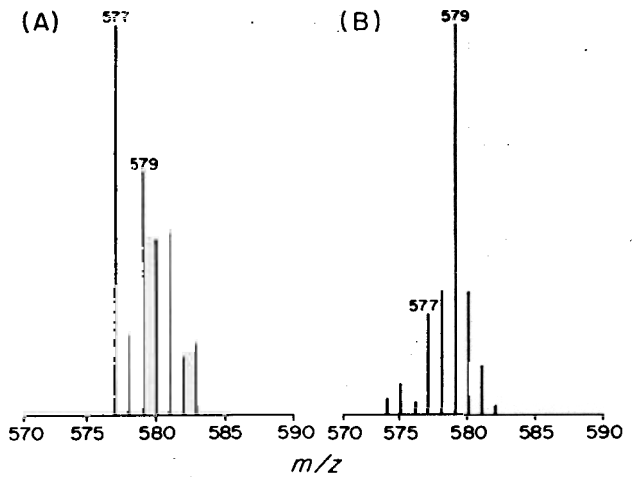


Figure 4. Dimeric regions of the mass spectra of (A) a linear trimer (V) and (B) a branched trimer (IV).

branched trimer (IV), cleavage of bond ii can only lead to formation of a carbonium ion at C4 of ring I, which again results in an ion peak at m/z 289 (VII). The upper two flavanoid units released on cleavage of bond ii give an ion peak at m/z 579 corresponding to a protonated dimer. Thus, in the spectrum of the branched trimer (Figure 3), sequence ions are observed only at m/z 289 and 579. B/E linked-scan spectra show that both sequence ions, in addition to the RDA fragment at m/z 715, can occur directly from unimolecular gas phase decomposition of $(M + H)^+$. By contrast, cleavage of the linear trimers (V and VI) at the lower bond, ii, gives a carbonium ion at C4 of the middle flavanoid unit (ring F), which results in formation of a dimer moiety with m/z 577 (IX). Thus, sequence ions observed for linear trimers occur at m/z 289, 577, and 579. Portions of typical mass spectra containing the dimer fragments of a linear and a branched trimer are compared in Figure 4. Figure 2B illustrates the metastable decomposition pathways leading to sequence ions for the branched (IV) and linear (V and VI) trimers.

The above findings show that procyanidin oligomers can be sequenced by FAB-MS, that information can be obtained about the type and location of the interflavanoid bonds, and that linear and branched oligomers can be differentiated on the basis of their fragmentation products. This information complements that which can be obtained by ^1H and ^{13}C NMR spectroscopy and forms a basis for structural elucidation of higher molecular weight polymers.

All of the experiments conducted in this study were performed on chromatographically isolated and purified samples; however, it should be feasible to extend the use of FAB coupled with MS–MS to the direct analysis of procyanidin oligomers without recourse to chromatographic isolation of pure compounds. The potential of this powerful approach to mixture analysis has already been demonstrated on a number of biopolymer systems (*20*, *21*) and should be equally applicable to the sequencing of procyanidin polymers. This possibility is currently being investigated.

Registry No. I, 103751-05-9; II, 20315-25-7; III, 103732-18-9; IV, 103751-06-0; V, 79763-28-3; VI, 79813-67-5.

LITERATURE CITED

(1) Haslam, E. In *The Flavonoids: Advances in Research*; Harborne, J. B., Marby, T. J., Eds.; Chapman and Hall: London, 1982; pp 417–447.
(2) Brown, B. R.; Lisseter, S. G. *Phytochemistry*, in press.
(3) Haslam, E. *Phytochemistry* **1977**, *16*, 1625–1640.
(4) Delcour, J. A.; Vandenberghe, M. M.; Dondeyne, P.; Schrevens, E. L.; Wijnhoven, J.; Moerman, E. *J. Inst. Brew.* **1984**, *90*, 67–72.
(5) Kumar, R.; Singh, M. *J. Agric. Food Chem.* **1984**, *32*, 447–453.
(6) Butler, L. G.; Riedl, D. J.; Lebryk, D. G.; Blytt, H. J. *J. Am. Oil Chem. Soc.* **1984**, *61*, 916–920.
(7) Klocke, J. A.; Chan, B. C. *J. Insect Physiol.* **1982**, *28*, 911–915.
(8) Zucker, W. V. *Am. Nat.* **1983**, *121*, 335–365.
(9) Hemingway, R. W. In *Organic Chemicals from Biomass*; Goldstein, I. S., Ed.; CRC Press: Boca Raton, FL, 1980; pp 189–248.
(10) Pizzi, A. In *Wood Adhesives, Chemistry and Technology*; Pizzi, A., Ed.; Marcel Dekker: New York, 1983; pp 177–246.

(11) Hemingway, R. W.; Laks, P. E.; McGraw, G. W.; Krelbich, R. E. In *Proceedings IUFRO Conference, Forest Products Research International—Achievements and the Future*; Pretoria, South Africa, 1985; Vol. 17, pp 1–20.
(12) Howes, F. N. *Vegetable Tanning Materials*; Butterworths: London, 1953; pp 152–156.
(13) Stafford, H. A.; Lester, H. H.; Porter, L. J. *Phytochemistry* **1985**, *24*, 333–338.
(14) Hemingway, R. W.; Laks, P. E. *J. Chem. Soc., Chem. Commun.* **1985**, 746–747.
(15) Foo, L. Y.; Hemingway, R. W. *J. Chem. Soc., Chem. Commun.* **1984**, 85–86.
(16) Hemingway, R. W.; Karchesy, J. J.; McGraw, G. W.; Wielsek, R. A. *Phytochemistry* **1983**, *22*, 275–281.
(17) Hemingway, R. W.; Foo, L. Y. *J. Chem. Soc., Chem. Commun.* **1983**, 1035–1036.
(18) Nonaka, G.; Morimoto, S.; Nishioka, T. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2139–2145.
(19) Porter, L. J.; Newman, R. H.; Foo, L. Y.; Wong, H.; Hemingway, R. W. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1217–1221.
(20) *Tandem Mass Spectrometry*; McLafferty, F. W., Ed. Wiley-Interscience: New York, 1983.
(21) *Mass Spectrometry in the Health and Life Sciences*; Burlingame, A. L.; Castagnoli, N., Jr., Eds.; Elsevier: New York, 1985; Chapters 9, 10, 12–14.
(22) McLafferty, F. W.; Bockhoff, F. M. *Anal. Chem.* **1978**, *50*, 69–76.
(23) Haddon, W. F. In *High Performance Mass Spectrometry*; Gross, M. L., Ed.; American Chemical Society: Washington, DC, 1978; pp 97–119.
(24) Karchesy, J. J.; Hemingway, R. W. *J. Agric. Food Chem.*, in press.
(25) Hemingway, R. W.; Foo, L. Y.; Porter, L. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1209–1216.
(26) de Koster, C. G.; Heerma, W.; Dijkstra, G.; Niemann, G. J. *Biomed. Mass Spectrom.* **1985**, *12*, 596–601.
(27) Thompson, R. S.; Jacques, D.; Haslam, E.; Tanner, R. J. N. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1387–1399.
(28) Jacques, D.; Haslam, E.; Bedford, G. R.; Greatbanks, D. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2663–2671.

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